2001 New Zealand Science and Technology Postdoctoral Fellowship

Molecular approaches for hexavalent chromium bioremediation: Stanford Medical Center

David F. Ackerley
School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington

In 2001, after responding to an advertisement in *Nature*, I was offered a postdoctoral position in the laboratory of Professor A.C. Matin in the Microbiology and Immunology Department of Stanford University, with the following proviso. 'I will employ you for a year. If you can obtain a fellowship within that time you can stay; if not – well, you'd better be damn good.' Fortunately the FRST Science and Technology Postdoctoral Fellowship saved me from being put to the test! Otherwise this article might have been much shorter.

And so I found myself in the dry heat of Northern California – conditions that after ten or so happy but slightly soggy years at Otago University I was willing to acclimatise to. Stanford is located in the heart of Silicon Valley in the San Francisco Bay Area, and has been a key player in the high-tech industry that the region is most famous for. For example, SUN Microsystems started life as a Stanford University Network communications project; and Hewlett-Packard and Google (back when it was a noun rather than a verb) were also Stanford-derived. The Bay Area has also developed into one of the world's leading biotechnology hubs, with Stanford again playing an integral role. Stan Cohen of Cohen-Boyer fame (i.e. the first recombinant DNA experiment, which in very short order enabled cloning of the human insulin gene and the concomitant launch of the leading biotechnology company Genentech) is still in the Department of Genetics there; and indeed, the Life Sciences academic staff member who is not on the board of, or consulting for, one or more biotech companies in the area is probably the exception rather than the rule. The desire of these companies to be affiliated with Stanford really reflects the top-notch work that so many of these scientists are doing. In my building alone were father and son Arthur and Roger Kornberg, Nobel prize winners in Medicine and Chemistry, respectively (it turns out that the research Roger was doing when he used to yell down the atrium at us to '…for God's sake SHUT UP' during Friday happy hours actually was quite important); Lubert Stryer, whom I met at one such happy hour and who was quite bewildered to hear that one of my fellow PhD students back at Otago had taken his biochemistry text-book on a world tour complete with photographs at the Eiffel Tower, the Colosseum, etc; and Stan Falkow, often referred to as the father of molecular microbial pathogenesis, who recently won the 2008 Lasker-Koshland Award for Special Achievement in Medical Science (perhaps the top Biology award outside of the Nobels) and who used to love talking about his annual holidays fly fishing in Nelson. All in all, it was a very vibrant and exciting scientific environment to suddenly be immersed in.

My postdoctoral research aimed to study and enhance the ability of environmental bacteria to 'bioremediate' hexavalent chromium (Cr (VI)), the toxic pollutant featured in the movie Erin Brockovich. For those unfamiliar with it, the movie is based on a civil class action lawsuit brought against the energy company Pacific Gas and Electric, who were sued for dumping Cr (VI) (which they used as a rust inhibitor in gas compressor cooling towers) into unlined ponds in the Mojave Desert, near the small township of Hinkley. Because Cr (VI) is extremely water-soluble it was able to migrate into the aquifer comprising the community's major water supply and consequently the residents suffered 'many physical ailments, including bloody noses, various intestinal ailments, bad backs, rotten teeth and tumors' (Sharp 2000). However, the effects of oral exposure to hexavalent chromium are poorly established and the underlying science, which was already a matter of some debate, was – inevitably – further muddied during the trial. In an extreme example, one paper (Zhang & Li 1997) that was a key piece of evidence for Pacific Gas and Electric was recently retracted by the journal on the basis of undisclosed 'financial and intellectual input to the paper by outside parties' (Brandt-Rauf 2006). Adding to the intrigue, the same researchers had previously interpreted the same data quite differently, suggesting that there was in fact a positive correlation between Cr (VI) ingestion and stomach cancer (Zhang & Li 1987). Nonetheless, because stomach acid is able to instantly reduce Cr (VI) to the non-bioavailable form Cr (III), it is not immediately clear how the polluted water might have caused the various disorders (although one expert witness, whom I spoke to informally at a conference, has suggested that inhalation of contaminated steam in the shower may have constituted an important route of exposure).

Although the movie did not really feature any science at all, that did not stop me littering my research posters and seminars

David Ackerley is a Senior Lecturer in the School of Biological Sciences at Victoria University of Wellington.

David's current research interests are: applications of bacterial oxidoreductase enzymes, in particular in anti-cancer gene therapy; and secondary metabolite synthesis by non-ribosomal peptide synthetase enzymes. He may be contacted at david.ackerley@vuw.ac.nz

with gratuitous shots of Julia Roberts and the original Erin (herself a former beauty queen), and the publicity also did not seem to hurt our various grant applications. Apart from FRST, our work was funded primarily by the US Department of Energy, who generated – and are now trying to address – a number of heavily Cr (VI)-contaminated sites, primarily as a by-product of nuclear weapons manufacture during the arms-race era. Without human intervention, Cr (VI) has been projected to persist at dangerous levels at such waste sites for well over 1000 years (Okrent & Xing 1993). Similar to the stomach acid example above, strategies for decontamination of environmental chromate focus on reducing it to non-bioavailable Cr (III). Chemical methods for this are prohibitively expensive for large-scale environmental application and frequently have damaging consequences of their own (Cervantes *et al*. 2001), and so bacterial bioremediation is of considerable interest as an environmentally friendly and affordable solution to chromate pollution. The main problem here is that, although many types of bacteria have the ability to reduce Cr (VI) to Cr (III), none of them seem to do it particularly well. This may be partly because Cr (VI) is a fairly recent anthropogenic pollutant, and bacteria simply have not yet evolved efficient systems for converting it. However, much of our research (as well as that of other groups) has also indicated that Cr (VI) reduction unavoidably entails the generation of toxic intermediates that are deleterious to the remediating organism (Keyhan *et al*. 2003; Ackerley *et al*. 2004a, b; Ackerley *et al.* 2006). The effects of this were particularly evident in some chromate-challenged *Escherichia coli* cell preparations that I once sent to a friend for electron micrograph; he quickly called me back to tell me that my samples must have become contaminated, as the Cr (VI)-treated one had been taken over by some kind of crazy filamentous microbe – or possibly by silly putty – but in fact these were the same *E. coli* cells three hours post-challenge, which had continued to elongate but had shut down cell division as a stress response (Figure 1).

We hoped to use genetic and protein-engineering strategies to maximise chromate reduction while minimising toxicity to remediating cells. Professor Matin had previously conducted some ground-breaking research into trichloroethylene (TCE) bioremediation, in which he had shown that placing a TCE-converting gene under control of a 'starvation promoter' was an effective way of maximising remediation under low-nutrient field conditions (Matin *et al*. 1995), and he felt that a similar strategy might work well with Cr (VI). Meanwhile, I was dead keen to try my hand at directed evolution, an exciting series of random mutagenesis techniques recently developed in California for improving enzyme activity with particular substrates of interest (Chen & Arnold 1993; Stemmer 1994). These ideas were quite complementary, both requiring identification and isolation of a gene encoding an enzyme with at least some Cr (VI)-reducing ability. To cut a long and fairly technical story short, we ended up identifying two different families of Cr (VI)-reducing enzyme and showed that they both contributed to Cr (VI) reduction by the host cell. Both types of enzyme were able to carry out a full conversion through to Cr (III), generating different levels of toxic intermediates and reactive oxygen species in the process (Gonzalez *et al*. 2003; Ackerley *et al*. 2004a, b). Consistent with the idea that Cr (VI) reduction is likely a 'promiscuous' property, we demonstrated that the primary biological role of at least one of these enzymes is probably defence against oxidative stress (Gonzalez *et al*. 2005) – and this was highly encouraging, as it suggested that this same enzyme might be able to mop up some of its own mess during Cr (VI) reduction. As it was also the enzyme that appeared to generate the fewest toxic chromium intermediates in the process, it was the logical candidate for our directed evolution schemes, aiming to generate a pimped up Cr (VI)-reducing super-enzyme. From one perspective, these efforts were actually quite successful – we ultimately managed to evolve an enzyme with 300-fold improvement in k_{cat}/K_m (a measure of its Cr (VI)-reducing efficiency) (Barak *et al*. 2006a). Unfortunately, however, when we actually popped this souped-up enzyme back into bacterial cells it transpired that we had now outstripped the ability of these cells to internalise Cr (VI); and consequently we only saw a modest 2- to 3-fold increase in the total rate of Cr (VI) reduction. Ongoing efforts in the Matin laboratory are now aimed at addressing the bacterial Cr (VI) uptake issue prior to any starvation promoter work and assessment of potential bioremediation utility.

Being the man I am, I was happy to leave this as 'Someone Else's Problem'. At the end of 2004 I was offered a position at Victoria University of Wellington (VUW), which, after some negotiation of the formal start date, I was pleased to accept. For reasons both scientific and personal (Figure 2), I was keen to stay on at Stanford for another year; and as long as I came back to New Zealand to help establish their new teaching programme in biotechnology and give a whirlwind flurry of lectures, VUW seemed happy to accommodate me. FRST were also enormously supportive during this process, allowing me to apply for (and

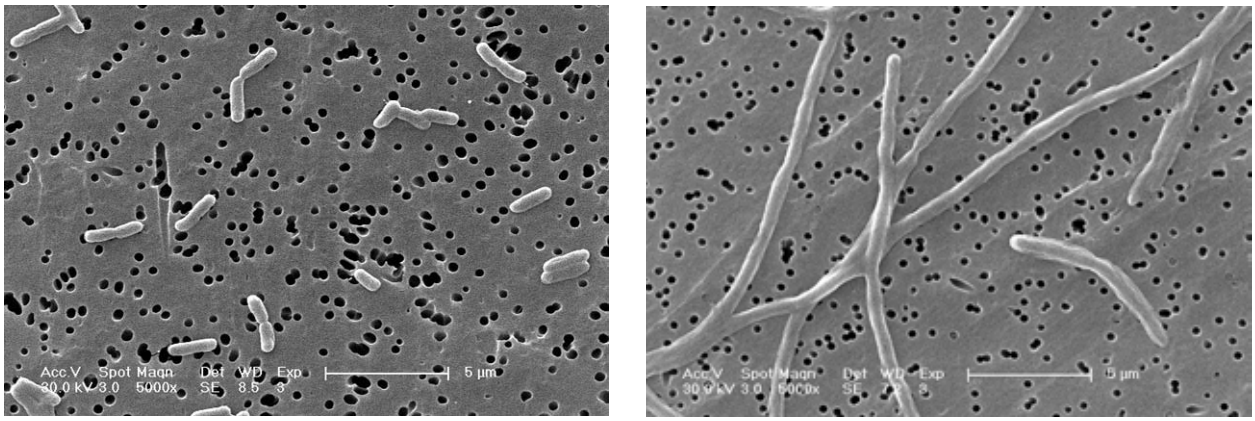

Figure 1: Representative scanning electron micrographs of* Escherichia coli *W3110 cells grown for 3 hours in* (left) *Luria Broth; or* (right) *Luria Broth amended with 250 µM K_2CrO_4.

Figure 2: Dave and his major scientific discovery at Stanford – wife Joanna MacKichan, who completed her PhD in 2004 in Stan Falkow's laboratory (it wasn't really the fishing stories that Dave was after) and is now investigating the molecular mechanisms of pathogenesis of* Neisseria meningitidis *at ESR (Institute of Environmental Science and Research Ltd).

ultimately granting) Bridge to Employment funding slightly outside of the usual dates. Not only did this award doubtless make my candidacy more attractive to VUW, it also provided them with some flexible funds to assist with my laboratory start-up package; and I am grateful to both FRST and VUW for their generosity in this regard.

At VUW I am continuing to work on the characterisation, engineering, and evolution of potentially useful bacterial enzymes, in particular following an interesting offshoot from the chromate project, where we showed that the same (wildly promiscuous!) enzymes are able to activate a variety of anti-cancer prodrugs (Barak *et al*. 2006b). This anti-cancer gene therapy potential has led to a very exciting multi-disciplinary collaboration with an amazingly talented group of molecular biologists and medicinal chemists at the Auckland Cancer Society Research Centre, in particular Drs Adam Patterson, Jeff Smaill, and Mike Hay, and Professors Bill Wilson and Bill Denny. This collaboration and an association with the Maurice Wilkins Centre for Molecular Biodiscovery have helped me obtain funding for the seven postgraduate students who are presently working in my laboratory; and I have even been lucky enough to get two postdoctoral research fellows of my very own.

Overall, I simply cannot emphasise enough how valuable the Science and Technology Postdoctoral Fellowships Scheme has been to my career. Without it, there may or may not have been funding for me to stay on at Stanford after my first (relatively unsuccessful) year was up; and while there were no hard-and-fast demands for me to come back to New Zealand at the end, the Scheme and the associated Bridge to Employment funding do certainly provide incentives to return. I found FRST to be very supportive and easy to work with, and, as a direct contact, Christine Romanes in particular was extremely helpful. To paraphrase Stuart McCutcheon's comment in the previous issue of this publication, the only reasonable criticism of the Fellowships is also a measure of their outstanding success – that it would be nice to have more of them. The Foundation does provide the only major source of postdoctoral research funding in New Zealand outside of individual grants; and now that I am on the other side of the academic fence I am better able to appreciate the enormous value and benefit that a talented postdoctoral research fellow can bring to a research laboratory. Not only do 'postdocs' have better training and higher skill levels than graduate students, they have the flexibility to work on and contribute to multiple projects without being bound by thesis constraints – and this type of coordination and independence can really help a laboratory transition from a collection of individuals into a unified research group. But they are awfully expensive… and therein lies the problem. I appreciate that funding is always limited, and lines must be drawn. It does, however, seem that the Fellowships are getting ever harder to obtain, and that people who are a lot more qualified than I was back in 2001 are missing out. I guess my point, ultimately, is that my Science and Technology Postdoctoral Fellowship provided one of the most valuable and formative experiences of my life, and it would be great to see more people having those opportunities.

In conclusion, I would like to thank FRST one more time for giving me that opportunity. Also Professor A.C. Matin and my co-workers in his laboratory, in particular Claudio Gonzalez, Yoram Barak, and Sue Lynch – thanks guys, I had a blast.

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